Genome Wide Analysis of Gene Silencing in Mammalian Cell Hybrids

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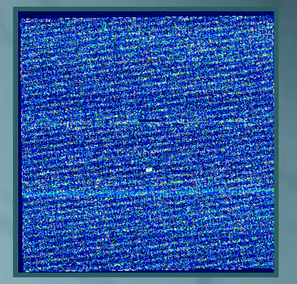

Abstract

For about 40 years it has been known that the fusion of mammalian cells of distinct origin produce hybrid cells that result in global loss of tissue-specific gene expression. Despite a great deal of research on this phenomenon, the mechanism of this process remains elusive. Due to recent advancements in microarray technology, it is possible to monitor gene expression of entire genomes in cell fusion experiments. We utilized microarray analysis to observe whole rat genome expression in rat hepatoma cells, rat fibroblast cells, and rat hepatoma x fibroblast hybrid cells. We used Rat Genome 230 2.0 array chips from Affymetrix which were incubated with labeled cDNA molecules derived from RNA extracted from each cell type using a Qiagen RNeasy kit, and then samples read on a chip reader. Preliminary results suggest that, in agreement with previous data, a large number of liver-enriched genes are moderately (5-10 fold) to strongly (>10 fold) repressed (194 and 300 genes, respectively) in the cell hybrids. A nearly equal number of fibroblast-specific genes were also repressed in the hybrid cells. Furthermore, 35 genes were activated >5 fold in the cell hybrids compared to either parental cell line. Thus, gene silencing in cell hybrids is bi-directional and affects a large portion of parental genomes. Also, a number of previously silent genes are activated in cell hybrids, some of which may be involved in the extensive gene silencing phenotype observed in cell hybrids.

Introduction

Tissue-specific gene expression has been studied by examining silencing of expression in somatic cell hybrids(1). In hepatoma x fibroblast cell hybrids, most of hepatic specific genes are silenced (2). Although in some cases this is reversible, it begs the question as to what mechanism the cells are using to produce the silencing of tissue-specific gene expression (2). Despite the fact that this phenomenon is poorly understood, it does appear that the causation of the phenotype is at the level of transcription (3). With the advent of microarray technology, we are able to observe genome-wide expression in parental cells and cell hybrids, and identify gene products that may contribute to silencing of tissue-specific gene expression.

Methods

Rat fibroblast cells (Rat1), hepatoma cells (FTO2B) and fibroblast x hepatoma hybrid cells (FR(2)) RNA was extracted from confluent monolayers the using an RNeasy kit from Qiagen. The RNA was analyzed by gel electrophoresis for visual integrity and relative concentration. The RNA samples were then sent to the W.M. Keck Center and Functional Genomics at University of Illinois in Urbana-Champaign. cDNA was synthesized from the RNA of each cell type via reverse transcriptase polymerase chain reaction (PCR), labeled and hybridized to the Affymetrix Rat Genome 230 2.0 Array chip and read on a chip reader. Two chips were used for each cell type.

Results

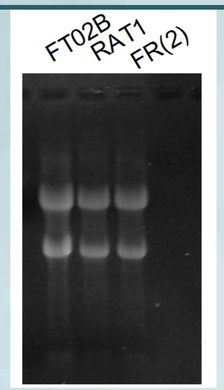

Figure 1. Gel analysis (MOPS) showed integrity of RNA. 1 x 10^7 cells (obtained by trypsinization) were lysed with 600ul RLT, and homogenized using a Qiashredder column. RNA was isolated using an RNAeasy kit, with the modification of a DNaseI step (15 minutes, RT) .

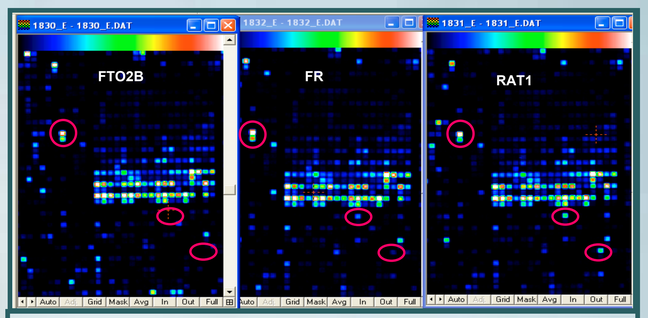

Figure 2. Example of readout of differential gene expression in hepatoma (FTO2B), fibroblast (Rat1) and cell hybrids (FR(2)) on the microarrays. Spots circled in lower area are expressed preferentially in the Rat1 cells.

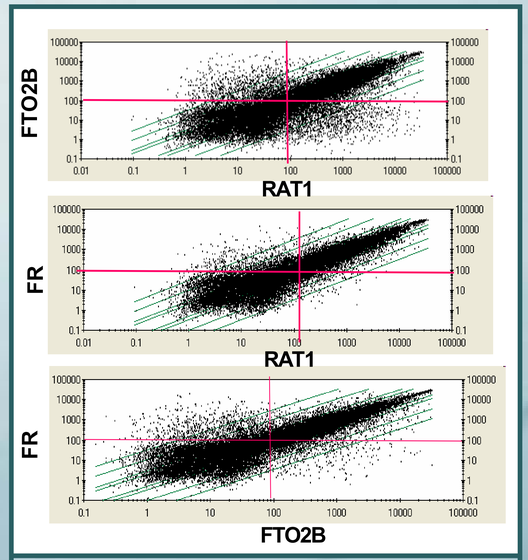

Figure 3. A comparison of genome-wide expression between hepatoma, fibroblast and cell hybrids.

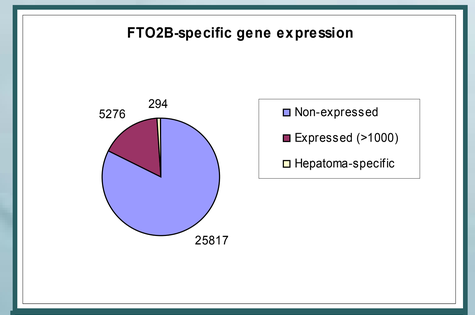

Figure 4. A comparison of total gene expression between hepatoma and fibroblast cells reveals 294 liver-specific genes (expressed >5 fold higher compared to fibroblasts levels).

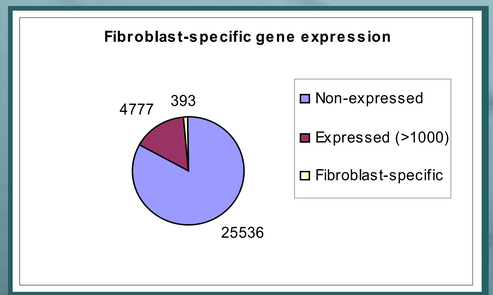

Figure 5. A comparison of total gene expression between hepatoma and fibroblast cells reveals 393 fibroblast-specific genes (expressed >5 fold higher compared to hepatoma levels).

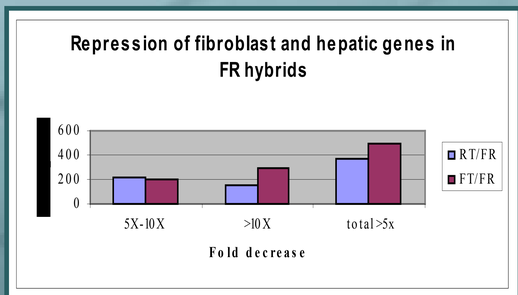

Figure 6. Large scale repression of both hepatoma-specific and fibroblast-specific genes were observed in the hybrid cells.

Discussion

- A large number of liver-enriched genes are moderately (5-10 fold) to strongly (>10 fold) repressed (194 and 300 genes, respectively) in the cell hybrids
- A nearly equal number of fibroblast-specific genes were also repressed in the cell hybrids
- 35 genes were activated >5 fold in the cell hybrids compared to either parental cell line
- Gene silencing in cell hybrids is bi-directional and affects a large portion of parental genomes
- A number of previously silent genes are activated in cell hybrids, some of which may be involved in the extensive gene silencing phenotype observed in cell hybrids.
- Subsequent investigation on candidate genes that may identify genes involved in tissue-specific gene silencing

Acknowledgements

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References

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